types of different linking oligonucleotides and nanoparticles used, and the strandedness of the oligonucleotide connectors, nanomaterials and nanostructures having a wide range of structures and properties can be prepared. These structures and properties can be varied further by cross-linking of the oligonucleotide connectors, by functionalizing the oligonucleotides, by backbone, base or sugar modifications of the oligonucleotides, or by the use of peptide-nucleic acids.

The nanomaterials and nanostructures that can be made by the nanofabrication method of the invention include nanoscale mechanical devices, separation membranes, bio-filters, and biochips. It is contemplated that the nanomaterials and nanostructures of the invention can be used as chemical sensors, in computers, for drug delivery, for protein engineering, and as templates for biosynthesis/nanostructure fabrication/directed assembly of other structures. See generally Seeman et al., *New J. Chem.*, **17**, 739 (1993) for other possible applications. The nanomaterials and nanostructures that can be made by the nanofabrication method of the invention also can include electronic devices. Whether nucleic acids could transport electrons has been the subject of substantial controversy. As shown in Example 21 below, nanoparticles assembled by DNA conduct electricity (the DNA connectors function as semiconductors).

Finally, the invention provides methods of making unique nanoparticle-oligonucleotide conjugates. In the first such method, oligonucleotides are bound to charged nanoparticles to produce stable nanoparticle-oligonucleotide conjugates. Charged nanoparticles include nanoparticles made of metal, such as gold nanoparticles.

The method comprises providing oligonucleotides having covalently bound thereto a moiety comprising a functional group which can bind to the nanoparticles. The moieties and functional groups are those described above for binding (*i.e.*, by chemisorption or covalent bonding) oligonucleotides to nanoparticles. For instance, oligonucleotides having an alkanethiol or an alkanedisulfide covalently bound to their 5' or 3' ends can be used to bind the oligonucleotides to a variety of nanoparticles, including gold nanoparticles.

The oligonucleotides are contacted with the nanoparticles in water for a time sufficient to allow at least some of the oligonucleotides to bind to the nanoparticles by means of the functional groups. Such times can be determined empirically. For instance, it has been found that a time of about 12-24 hours gives good results. Other suitable conditions for binding of the oligonucleotides can also be determined empirically. For instance, a concentration of about 10-20 nM nanoparticles and incubation at room temperature gives good results.

Next, at least one salt is added to the water to form a salt solution. The salt can be any water-soluble salt. For instance, the salt may be sodium chloride, magnesium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, a combination of two or more of these salts, or one of these salts in phosphate buffer. Preferably, the salt is added as a concentrated solution, but it could be added as a solid. The salt can be added to the water all at one time or the salt is added gradually over time. By "gradually over time" is meant that the salt is added in at least two portions at intervals spaced apart by a period of time. Suitable time intervals can be determined empirically.

The ionic strength of the salt solution must be sufficient to overcome at least partially the electrostatic repulsion of the oligonucleotides from each other and, either the electrostatic attraction of the negatively-charged oligonucleotides for positively-charged nanoparticles, or the electrostatic repulsion of the negatively-charged oligonucleotides from negatively-charged nanoparticles. Gradually reducing the electrostatic attraction and repulsion by adding the salt gradually over time has been found to give the highest surface density of oligonucleotides on the nanoparticles. Suitable ionic strengths can be determined empirically for each salt or combination of salts. A final concentration of sodium chloride of from about 0.1 M to about 1.0 M in phosphate buffer, preferably with the concentration of sodium chloride being increased gradually over time, has been found to give good results.

After adding the salt, the oligonucleotides and nanoparticles are incubated in the salt solution for an additional period of time sufficient to allow sufficient additional oligonucleotides to bind to the nanoparticles to produce the stable nanoparticle-

oligonucleotide conjugates. As will be described in detail below, an increased surface density of the oligonucleotides on the nanoparticles has been found to stabilize the conjugates. The time of this incubation can be determined empirically. A total incubation time of about 24-48, preferably 40 hours, has been found to give good results (this is the total time of incubation; as noted above, the salt concentration can be increased gradually over this total time). This second period of incubation in the salt solution is referred to herein as the "aging" step. Other suitable conditions for this "aging" step can also be determined empirically. For instance, incubation at room temperature and pH 7.0 gives good results.

The conjugates produced by use of the "aging" step have been found to be considerably more stable than those produced without the "aging" step. As noted above, this increased stability is due to the increased density of the oligonucleotides on the surfaces of the nanoparticles which is achieved by the "aging" step. The surface density achieved by the "aging" step will depend on the size and type of nanoparticles and on the length, sequence and concentration of the oligonucleotides. A surface density adequate to make the nanoparticles stable and the conditions necessary to obtain it for a desired combination of nanoparticles and oligonucleotides can be determined empirically. Generally, a surface density of at least 10 picomoles/cm^2 will be adequate to provide stable nanoparticle-oligonucleotide conjugates. Preferably, the surface density is at least 15 picomoles/cm^2. Since the ability of the oligonucleotides of the conjugates to hybridize with nucleic acid and oligonucleotide targets can be diminished if the surface density is too great, the surface density is preferably no greater than about 35-40 picomoles/cm^2.

As used herein, "stable" means that, for a period of at least six months after the conjugates are made, a majority of the oligonucleotides remain attached to the nanoparticles and the oligonucleotides are able to hybridize with nucleic acid and oligonucleotide targets under standard conditions encountered in methods of detecting nucleic acid and methods of nanofabrication.

Aside from their stability, the nanoparticle-oligonucleotide conjugates made by this method exhibit other remarkable properties. See, *e.g.,* Examples 5, 7, and 19 of the present